

Risk assessment for the deployment of a Costa Rican rice variety resistant to ammonium gluphosinate (PPT) in tropical America

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Prior to the release of Costa Rican transgenic rice varieties resistant to ammonium gluphosinate (PPT), an integrated risk assessment evaluation is required, to determine the potential environmental impact of gene flow to native wild *Oryza* species and the weedy rice complex. Populations of the wild relatives of rice were identified, located and characterized by CIBCM throughout the country. This study demonstrated that Costa Rica is home for three of the four wild *Oryza* species native to tropical America: *O. glumaepatula*, *O. latifolia* and *O. grandiglumis*. *O. glumaepatula* is the species more closely related to cultivated rice, *O. sativa*, sharing AA-type genome, while the others are allotetraploid, with CCDD genomes. The genetic relationship of *O. glumaepatula* and the cultivated rice raises concerns about gene flow from cultivated rice to these natural populations, particularly, in the Medio Queso wetlands located in the north part of Costa Rica, where the two species grow in near proximity. In order to fill the gap on knowledge about the reproductive biology of *O. glumaepatula*, progeny studies were performed using molecular markers. *O. glumaepatula* mother plants and their progenies were analyzed using fluorescent microsatellites, demonstrating that *O. glumaepatula* is predominantly autogamous but occasionally behaves as a facultative allogamous species. To study the compatibility between *O. glumaepatula* and *O. sativa*, artificial crosses were performed between both species. The hybrid nature of F1 seeds was confirmed using fluorescent microsatellites. In addition, the hybrid seeds obtained were planted in greenhouse and twelve morphological traits were evaluated. As expected, the morphology of the hybrids *glumaepatula-sativa* showed intermediate characters but they were sterile.

The weedy rice complex is another potential recipient of genes if genetically modified rice lines are deployed in the field because it is genetically related and grows sympatrically with *O. sativa*. An inventory of weedy rice complex in the country was carried out for their morphologic, phenologic and molecular characterization. The hypothesis was that the weedy rice morphotypes could result from hybridization events among commercial rice varieties, old landraces, native wild *Oryza* species of the Americas and introduced wild rice species of Asia and Africa (*O. rufipogon* and *O. glaberrima*). The morphometric relationships of the weedy rice accessions were established by comparing 27 morphological traits with commercial rice cultivars, landraces and wild *Oryza* species, using a multivariate analysis. The majority of the weedy morphotypes, grouped closer to the commercial rice varieties, while others grouped with *O. rufipogon*. Additionally, a group showed intermediate characters between *O. sativa* and *O. rufipogon*, suggesting that hybridization could have taken place in the past between these species. None of the morphotypes collected in Costa Rica clustered either with *O. latifolia*, *O. grandiglumis* or *O. glumaepatula*. Molecular analyses using microsatellites are in progress in order to determine if the clusters previously described are maintained. Little was known about the phenology of the weedy rice morphotypes described before, and traits like days to reach anthesis are of particular interest when gene flow studies are carried out. Therefore, a field trial was established with the 21 Costa Rican weedy rice morphotypes, five commercial rice varieties and accessions of *O. glaberrima* and *O. rufipogon* to study the vegetative and reproductive cycle of these materials. Seventy one percent of the weedy types overlap flowering with the variety Setesa-9 (early variety), whereas 50% of weedy rice overlapped flowering with the varieties CR-5272 (intermediate variety) and CR-4338 (intermediate variety), and just one type- overlapped anthesis with CR-1821 (late variety). The previous results suggest that early and intermediate varieties are more prone to hybridize with weedy rice morphotypes under Costa Rican conditions.

Weedy rice accessions representing the diversity of the morphotypes found in the Costa Rican populations were selected to conduct gene flow studies in the field. Gene flow studies between rice and six weedy rice morphotypes were conducted either tracing the morphological marker anthocyanins (dominant trait) from the Costa Rican rice variety Setesa-9 or the herbicide PPT resistance trait from the transgenic rice variety. Field experiments were designed by mixing seeds of Setesa-9 (pollen donor plants) with each one of three weedy rice morphotypes selected to simulate infestation levels of 10%, 30% and 60%. In the

case of the nearly neutral anthocyanin (purple) marker gene, preliminary data on hybridization rates varied from 0.36% to 2.59% in approximately 10,000 progeny plants evaluated per each weedy morphotype. In contrast to field assays, manual-made crosses performed under greenhouse conditions between Setesa-9 and the weedy morphotypes showed significantly higher hybridization rates of 15% to 30% than the gene flow registered under natural conditions. In the case of the transgenic glufosinate resistance trait, rates of 0.03% to 0.72% were recovered in 4300-8000 progeny plants analyzed per weedy morphotype (using six different morphotypes and infestations levels of 10%, 15% and 30% and two applications of the herbicide PPT). Differences in outcrossing rates were observed among morphotypes but the infestations levels did not influenced gene flow rates. Putative hybrids will be confirmed using molecular techniques such as microsatellites in the case of Setesa-9 field trial and the *bar* gene by means of PCR in the case of transgenic plants.

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References

- Arrieta-Espinoza, G., Quesada, T., Gamboa, E., Sánchez, E., & Espinoza, A. M. (2002). Transgenic rice and gene flow assessment to wild and weedy rice species in Costa Rica. In abstracts of the OECD International Conference LMOS and the Environment, held in Raleigh, North Carolina, USA. Edited by C.R. Roseland, pp 69-79.
- Arrieta-Espinoza, G., Sánchez, E. Lobo, J. & Espinoza, A. M. (2004). The weedy rice complex in Costa Rica. I.Morphologic study and relationships with commercial rice varieties and wild *Oryza* relatives. Genetic Resources and Crop Evolution (Accepted).
- Espinoza, A. M. (2000). Transgenic rice in Costa Rica: A study case for biosafety considerations. In: abstracts of the international congress "Perspectives and limitations of biotechnology in developing countries" (Full paper). Organized by the University of Costa Rica and the European Community), held in San José, Costa Rica.
- Espinoza, A. M., Sittenfeld, A., & Salazar, S. (2003). Developing of transgenic rice at the University of Costa Rica: Perspectives and considerations for managing intellectual property rights. *Interciencia* 28:111-117.
- Espinoza, A.M. and Arrieta-Espinoza, G (2004). Liberation of Costa Rican transgenic rice to consumers: experiences beyond the laboratory. In abstracts of the V Encuentro Latinoamericano y del Caribe de Biotecnología Agrícola de la RebBio, Dominican Republic.
- Quesada, T., Lobo, J. & Espinoza, A. M. (2002). Genetic diversity and mating system of the wild rice species *Oryza latifolia* Desv. *Genetic Resources and Crop Evolution* 49:633-643.
- Quesada, T., Lobo, J. & Espinoza, A.M. (2003). Biología reproductiva de la especie silvestre de arroz *Oryza glumaepatula* Steud. determinada mediante análisis de progenies. In abstract of the Jornadas de Académicas de la Universidad de Costa Rica.
- Zamora, A. (2001). Diversidad morfológica y genética de las especies de *Oryza* (poaceae) nativas de Costa Rica. Tesis Magister Scientiae in Biology. Universidad de Costa Rica. 178p.
- Zamora-Meléndez, A., González, P. & Espinoza, A. M. (2003). Wild rice (Poaceae: *Oryza*) species of Costa Rica: diversity and distribution. *Genetic Resources and Crop Evolution* 50:855-870.